

Fig. 1

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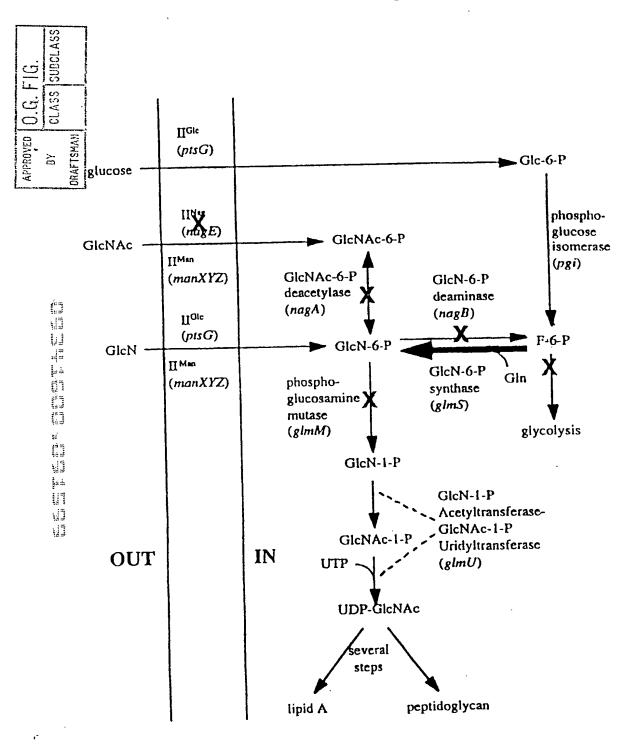
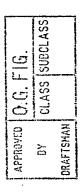


Fig. 2
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Strain Construction Scheme

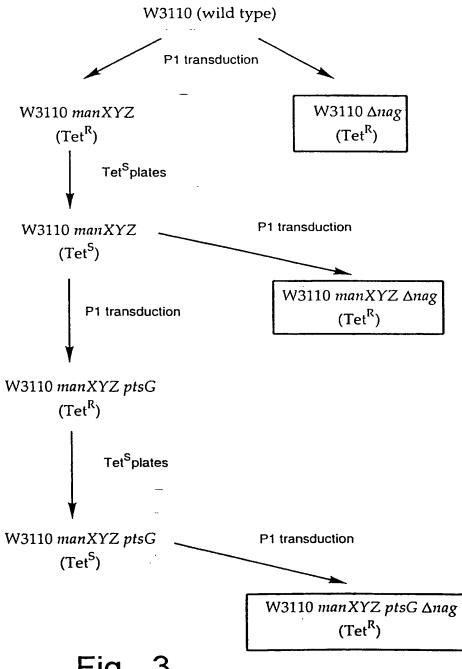


Fig. 3

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## Effects of Initial Glucose Concentrations and Glucose Feeds on Glucosamine Production

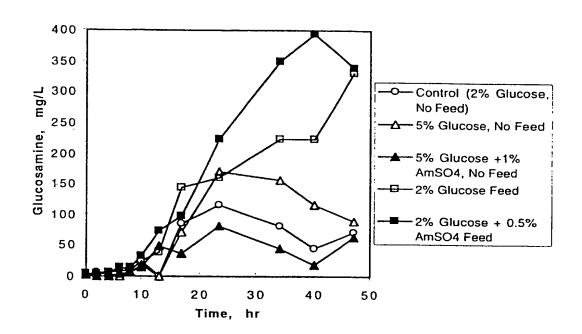


Fig. 4

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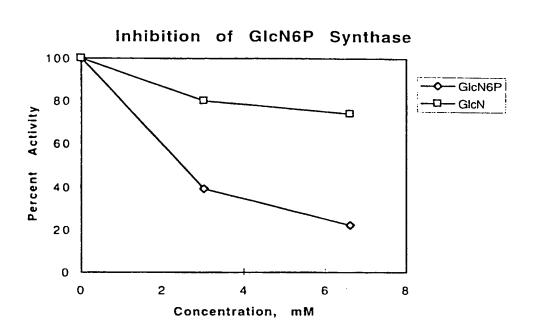


Fig. 5

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Product Inhibition of Glucosamine 6-phosphate Synthase in Mutant glmS Clones

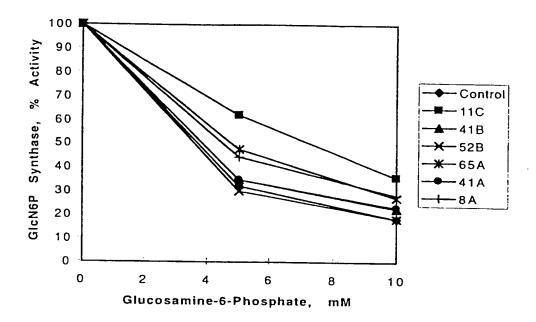


Fig. 6

## Construction of Strains Containing Mutant glmS Genes

pKLN 23-28

1) Error-prone PCR

mutant glmS genes

2) Clone into pKLN23-28 background

library of integration plasmids containing mutant glmS genes

3) Transfer to strain 7101-17(DE3)

plasmid-containing strains with mutant glmS genes in production background

4) Cross-feeding assay with glmS mutant indicator strain

large "halo" producing strains

- 5) Isolate plasmid DNA
- 6) Transfer to ATCC 47002
- 7) Screen for integration into chromosome

mutant T7-glmS in chromosome

8) Transfer mutated genes to 7101(DE3) by P1 transduction

production strains with mutant T7-glmS in chromosome

Fig. 7

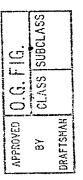
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Product Inhibition of Glucosamine 6-phosphate Synthase in Strains with Integrated Mutant glmS Genes

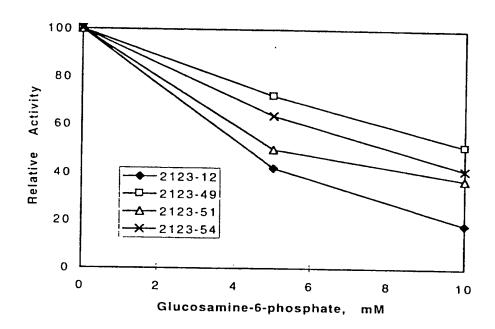


Fig. 8

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Glucosamine Production in Strains with Integrated Mutant glmS Genes

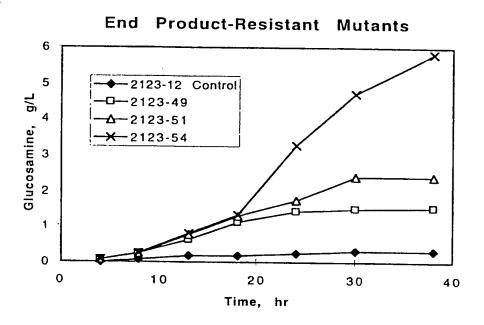


Fig. 9